RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

Design, Synthesis and Biological Evaluation of Novel Podophyllotoxin Derivatives Bearing 4β-Disulfide/trisulfide Bond as Cytotoxic Agents †

meiReceived 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Shi-Jun Zhu[+], Hua-Zhou Ying[+], Yan Wu, Ni Qiu, Tao Liu, Bo Yang, Xiao-Wu Dong*, Yong-Zhou Hu*

A novel series of C-4β-disulfide/trisulfide-containing podophyllotoxin derivatives were designed, synthesized, and biologically evaluated for their cytotoxic activities against human cancer cell lines, including KB (Mouth Epidermal Carcinoma Cells)and KB/VCR (Vincristine-resistantMouth Epidermal Carcinoma Cells). Most of these compounds exhibited promising moderate to good cytotoxic activities. In particular, some of them displayed even superior activities to that of etoposide, especially for KB/VCRcell lines, indicating that introduction of disulfide/trisulfide moiety would be benefical for overcoming the multi-drug resistant limitation of etoposide. Moreover, the metabolic evaluation of the most promising compound was further performed toreveal that difulfide bond can be stable in human plasma over 8 hours, indicating a good prospect of these compounds for in-vivo anti-cancer activities.

1 Introduction

Disulfide/trisulfide moiety (−S−S−/−S−S−S−) has been demonstrated to possess diverse functionalities, such as, switch for protein function,[1] conversion of reactive oxygen species ($O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$),[2] and crucial role in targeting tumors.[3] They frequently occurred in a variety of natural/synthetic compounds[4] (*i.e.* leinamycin,[5] thiarubrines,[6] varacin,[7] and esperamicins[8]) (Fig. 1A) and the emerging role of disulfide and multisulfide therapeutic agents have been well recognized. For instance, Vyas et. al.[9a] and kono et. al.[9b] reported the mitomycin disulfides **M-1a** and **M-1b**, which were modified from mitomycin C by replacing the C(7) amine with aminoethylene disulfide group. The resulting **M-1a** and **M-1b** exhibited superior biological profiles compared with mitomycin C, which represented 10~100-folds greater cytotoxicity in tumor cell lines and more efficient cellular uptake.

Etoposide (VP-16, Fig. 2), an analogue of podophyllotoxin, has been widely used for treatment of numerous solid tumors (e.g. lung, ovarian and testicular cancer) and hematological cancer (e.g. lymphoma).[10] Nevertheless, there still are some undesirable side effects (*e.g.* myelosuppression, neurotoxicity)

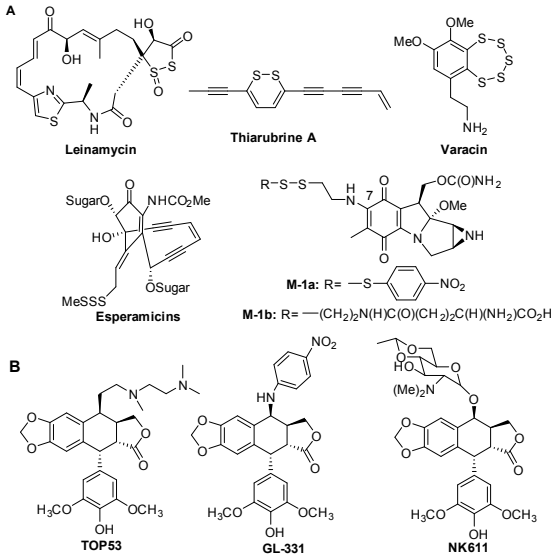


Fig.1. The structures of Disulfide/trisulfide-containing natural/synthetic compounds (A) and etoposide analogues (B).

and drug-resistance problems that have occurred in the clinical application.[11] With aim to address these existing problems, a number of etoposide derivatives have been designed and synthesized, subsequently, their structure-activities relationships were extensively explored, revealing that C-4 moiety on ring C of etoposide would be extremely tolerant for anti-cancer activities.[12] As a consequence, a variety of podophyllotoxinderivatives, such as TOP53,[13] GL-331[14] and NK611[15] were disclosed (Fig. 1B), some of which showed

[a] ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, People's Republic China
[b] *Corresponding authors. Tel. (Fax.): +86-571-88208460. E-mail: huyz@zju.edu.cn (Y.-ZHu). Tel. (Fax.): +86-571-88981051. E-mail: dongxw@zju.edu.cn (X.-W. Dong).
[+] These authors are equally contributed to this work.
†Electronic Supplementary Information (ESI) available: The synthesis of side chain intermediates and NMR spectra of the final products See DOI: 10.1039/x0xx00000x

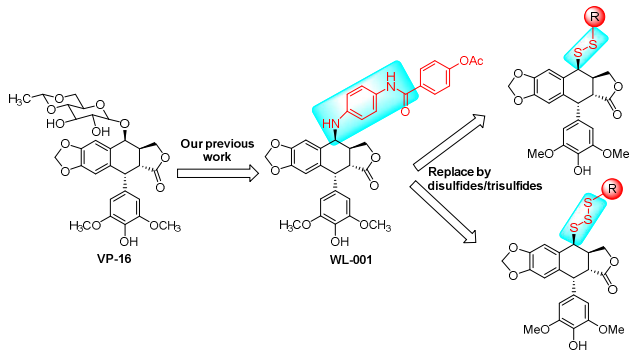


Fig.2.The design of disulfide/trisulfide-containing 4β-podophyllotoxin derivatives.

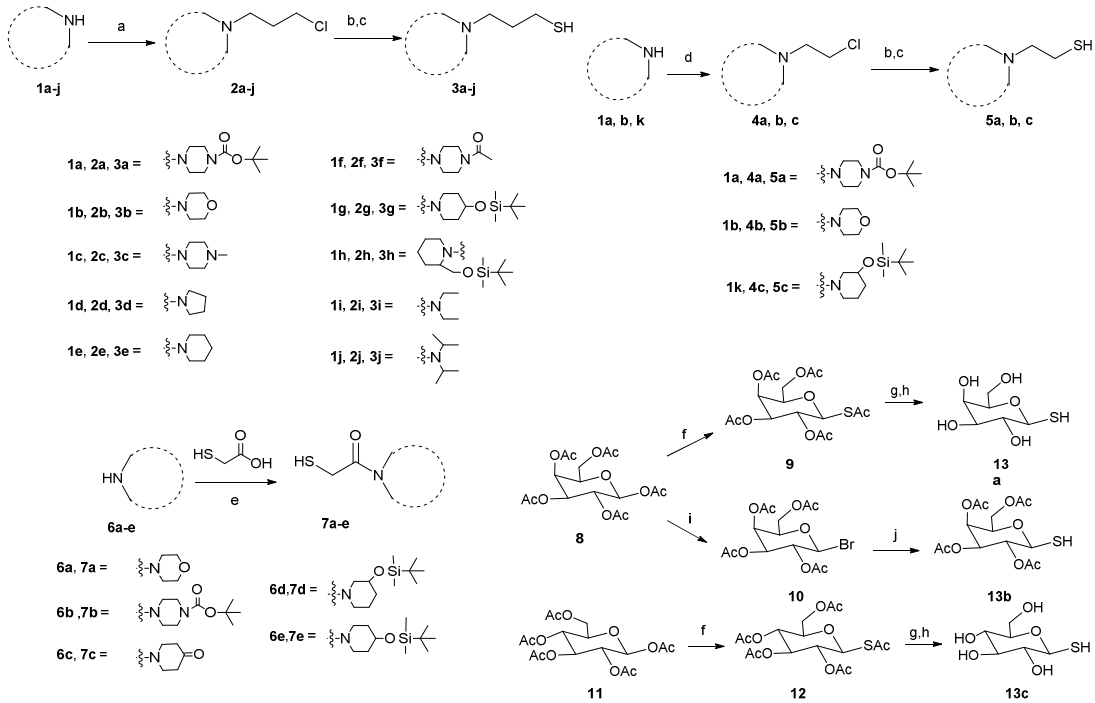
superior biological activities compared with etoposide, especially for multi-resistant cancer cells. In our previous study, we also reported a series of 4β-anilino substituted podophyllotoxin derivatives (e.g. **WL-001**, Fig. 2) with potent cytotoxic activities (IC_{50} = 1.91, 8.48 μM) against KB and KB/VCR cancer cells, respectively, which is superior to that of VP-16 (IC_{50} = 4.61, 83.4 μM).[16] Nevertheless, toxicity was observed during in-vivo study, thereby hindering the further development of these compounds and promoting us to discover other novel podophyllotoxin derivatives as anti-cancer agents. In this context, due to the multi-functionality and good biological compatibility of disulfide/trisulfide bonds, we envisioned that it would act as a promising alternative linker between podophyllotoxin and various C4-side chains (Fig. 2)

Herein, we designed and synthesized a variety of novel disulfide/trisulfide-containing 4β-podophyllotoxin derivatives (**17a-y**, **18a-e** and **22a-f**). To our knowledge, this is the first time disulphide/trisulfide moiety was introduced into podophyllotoxin. All of the prepared compounds were tested for their cytotoxic activities against KB and KB/VCR cell lines. Subsequently, their preliminary tructure-activity relationships (SARs) were explored in-depth to investigate the effect of the linker (i.e. disulfide and trisulfide) and various side chains. Moreover, the metabolism stability in the human plasma was carried out to evaluate the practical application value in further development of compound **17l**, which showed the most promise in in-vitro study.

2 Results and discussion

2.1 Chemistry

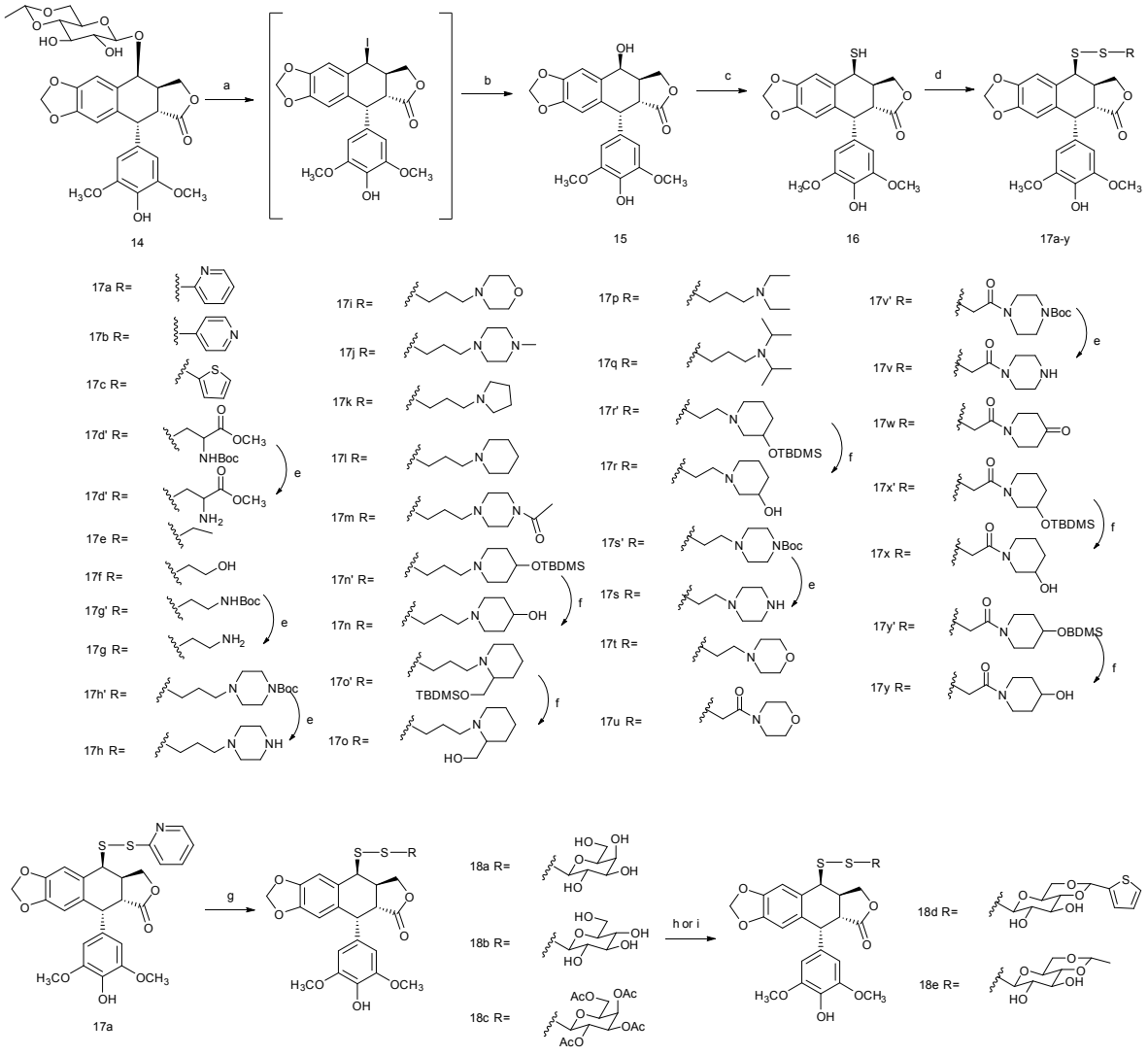
Considering that disulfide bonds can be efficiently formed by oxidation of two different free thiol compounds, the synthesis of disulfide-containing podophyllotoxin derivatives **17a-y** and **18a-e** were divided into two part, including thiol-containing side chain moieties **3a-j**, **5a-c**, **7a-e** and **13a-c** (Scheme 1) and 4β-mercapto-4'-O-demethyl-4-desoxypodophyllotoxin **15** (Scheme 2). Thiol compounds **3a-j** and **5a-c** were obtained via nucleophilic reaction of thiourea with chloride-substituted alkyl compounds **2a-j** or **4a-c**, which were acquired by the



Scheme 1 The synthetic route of thiol-containing sidechain moieties **3a-j**, **5a-c** and **7a-e** and **13a-c**. Reagents and conditions: (a)1-bromo-3-chloropropane, K_2CO_3, CH_3CN, r.t.; (b) thiourea, KI, EtOH, reflux; (c) NaOH aq. reflux; (d) 1-bromo-2-chloroethane, K_2CO_3, CH_3CN, r. t.; (e) HOBt, EDC, DCM; (f) thiolacetic acid, $BF_3{\cdot}Et_2O$; (g) CH_3ONa, CH_3OH; (h) cation exchange resin; (i) 33% HBr in AcOH, DCM; (j) $NaSH{\cdot}9H_2O$, CS_2, DMF.

Journal Name

ARTICLE

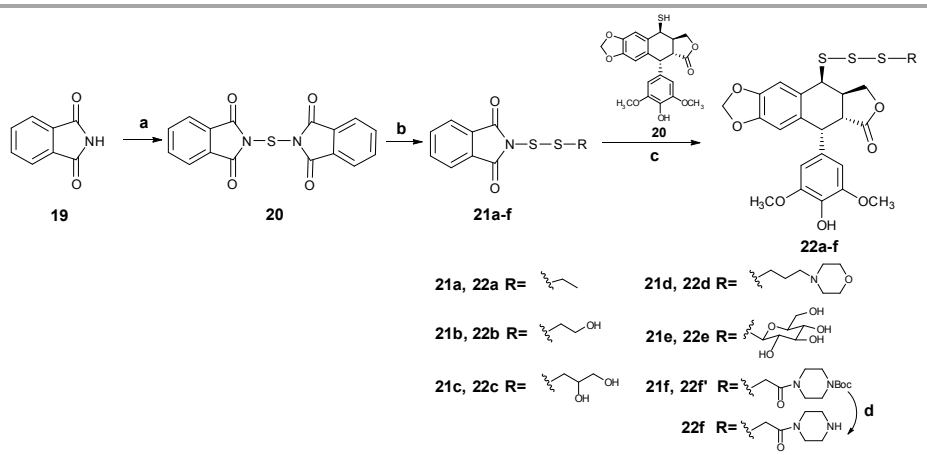


Scheme 2 The synthetic route of disulfide-containing 4β-podophyllotoxin derivatives **17a-y** and **18a-e**. Reagents and conditions: (a) NaI, TMSCl, CH_3CN, 0 °C; (b) $BaCO_3$, H_2O/Acetone, r.t.; (c) H_2S saturated DCM, $BF_3{\cdot}Et_2O$, pyridine, -15 °C; (d) 1-chlorobenzotriazole, benzotriazole, -78 °C,appropriate thiol-containing side chain moiety, DCM; (e) HCl saturated EtOAc, DCM; (g) $BF_3{\cdot}Et_2O$, CH_3CN; (f) $CHCl_3$, r.t.; (g) 2-thenaldehyde, $ZnCl_2$, N_2; (h) TsOH,1,1-Dimethoxyethane, acetonitrile.

substitution reaction of **1a**-**j** with 1-bromo-3-chloropropane/1-bromo-2-chloroethane, and successively alkali hydrolysis in presence of sodium hydroxide aqueous solution. On the other hand, compounds **7a**-**e** were prepared by directly condensation of mercaptoacetic acid with appropriate substituted amines **6a**-**e**. Preparation of glycosylthioacetates **9** and **12** were archived by reaction of per-O-acetylated sugar precursors **8** or **11** with boron trifluoride diethyl etherate and mercaptoacetic acid, respectively. Then, deprotection of **9** and **12** in presence of CH_3ONa/CH_3OH furnished thiol-containing sugars **13a** and **13c**, respectively. Similarly, the hydroxyl-acetylated thiol galactose **13b** was obtained by bromination of per-O-acetylated galactose **9** and then sulfurization by sodium hydrosulfide.

Journal Name

ARTICLE



Scheme 3 The synthetic route of trisulfide-containing 4β-podophyllotoxinderivatives **22a**-**22f**. Reagents and conditions: (a) S_2Cl_2, DMF; (b) DCM, appropriate thiol-containing side chain moiety, Et_3N, r. t.; (c) DCM, r. t.;(d) HCl saturated EtOAc, DCM.

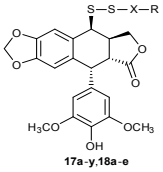
Meanwhile, the synthesis of 4β-hydroxy-4'-O-demethyl-4-desoxypodophyllotoxin **15** was furnished by the C4-iodination of etoposide, and subsequently hydrolysis in presence of barium carbonate. Then, 4β-mercapto-4'-O-demethyl-4-desoxypodophyllotoxin **16** was prepared with high stereospecificity by the reaction of **15** and H_2S in the presence of BF_3•Et_2O.[17] On one hand, target compounds **17a-c**, **17e**, **17f**, **17i-m**, **17p**, **17q**, **17t**, **17u**, **17w** and intermediates **17d'**, **17g'**,**h'**, **17n'**, **17o'**, **17r'**, **17s'**, **17v'**, **17x'**, **17y'** were obtained by oxidation of **16** with appropriate thiol compounds in dichloromethane.[18] And the protection groups (-Boc and -TBDMS) of the obtained intermediates were easily removed by HCl saturated EtOAc and BF_3•Et_2O, respectively, to afford target compounds **17d**, **17g**-**h**, **17n**, **17o**, **17r**, **17s**, **17v**, **17x** and **17y**. On the other hand, the sugar-containing target compounds **18a**-**c** were synthesized by reacting of thiol-containing sugars **13a**-**c** with 2-mercaptopyridine-activated 4β-mercapto-4'-O-demethyl-4-desoxypodophyllotoxin **17a**. Furthermore, compounds **18d**, **e** were obtained by acetalation of **18b** by thiophenecarboxaldehyde and acetaldehyde dimethyl acetal, respectively.

Besides, the synthesis of trisulfide-containing podophyllotoxin derivatives **22a**-**f** was illustrated in Scheme 3. Initially, the preparation of 2,2'-thiobis(isoindoline-1,3-dione) **20** was achieved according to the literature.[19] Then, N-disulfanylisoindolinediones **21a**-**f** were synthesized by the thiol exchange reaction of **20** with 4β-mercapto-4'-O-demethyl-4-desoxypodophyllotoxin **16**, and subsequently used to synthesize compounds **22a**-**e** and **22f'** via second thiol exchange reaction and deprotection if necessary.[20] And compound **22f** was obtained by deprotection of **22f'** in acidic condition.

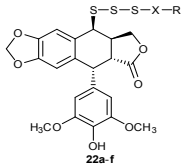
2.2 Cytotoxic activities against KB and KB/VCR cells

All of target compounds **17a-y**, **18a-e** and **22a-f** were tested for *in vitro* cytotoxic activities against KB and vincristine-resistant KB/VCR cell lines. The results of them together with etoposide are summarized in Table 1 and Table S1 (ESI†). In general, most of the synthesized disulfide/trisulfide derivatives exhibited moderate to potent cytotoxic activities, especially for KB cell lines, which were much better than that of etoposide. Comparing the cytotoxic activities against KB cell lines of disulfide and trisulfide series, in general, the disulfide derivatives **17a-y** and **18a-e** were more potent than trisulfide derivatives **22a-f**. As exemplified in compounds **17e** and **22a** with the same 4β-side chain, compound **17e** showed 2.7-folds more potent cytotoxic activity against KB cell than that of **22a**. Similar SAR rules were observed in comparison with **17i** and **22d**. Insight into the effect of different 4β-substitution (*e.g.* heteroaryl, alkyl, heteroalkyl and glycosyl) of podophyllotoxin on cytotoxic activities reveals that the compounds **17a-c** with heteroaryl group (*o*-pyridine, *p*-pyridine and *o*-thiophen) showed preferable activities, with IC_{50} value less than 1 μM (IC_{50} of **17a**, **17b**, **17c** against KB were 0.30, 0.17 and 0.82 μM, respectively), which were significantly more potent than compounds **17d-y** and **18a-e** with alkyl/heteroalkyl/glycosyl group at 4β-position. Although cytotoxic activities against KB cell were not affected by the length and rigidity of linker between the disulfide bond and amine moieties, for example, the compounds with *n*-propyl-linker (e.g.**17h** and **17i**) showed

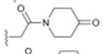
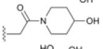
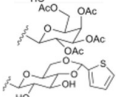
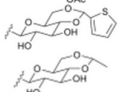
Table 1 Cytotoxic activities of target compounds against selected human cancer cell lines.



17a-y,18a-e



22a-f

Compd.	X	R	IC_{50} (μM) KB	KB/VCR	RF[a]
Etoposide	-	-	2.27±0.58	16.8±0.11	7.40
17a	-		0.3±0.05	9.1±0.67	30.3
17b	-		0.17±0.06	7.54±0.48	44.3
17c	-		0.82±0.11	5.2±2.72	6.34
17d	-		7.19±1.59	16.9±0.86	2.35
17e	$-(CH_2)_2-$	H	10.2±2.42	13.8±0.8	1.35
17f	$-(CH_2)_2-$	-OH	10.2±2.11	18.4±2.15	1.80
17g	$-(CH_2)_2-$	$-NH_2$	4.97±1.2	11.9±0.47	2.39
17h	$-(CH_2)_3-$		1.05±0.24	4.12±0.43	3.92
17i	$-(CH_2)_3-$		1.52±0.48	6.44±0.43	4.24
17j	$-(CH_2)_3-$		3.72±0.48	6.44±0.43	1.73
17k	$-(CH_2)_3-$		4.22±0.99	2.98±0.44	0.71
17l	$-(CH_2)_3-$		2.90±0.72	0.97±0.03	0.33
17m	$-(CH_2)_3-$		3.72±1.22	10.0±0.69	2.69
17n	$-(CH_2)_3-$		1.25±0.98	4.88±1.10	3.90
17o	$-(CH_2)_3-$		1.12±0.65	13.8±0.89	12.3
17p	$-(CH_2)_3-$		1.81±0.46	2.18±0.61	1.20
17q	$-(CH_2)_3-$		2.02±0.55	1.46±0.02	0.72
17r	$-(CH_2)_2-$		6.77±1.66	14.3±1.66	2.11
17s	$-(CH_2)_2--$		1.36±0.42	12.5±0.54	9.19
17t	$-(CH_2)_2-$		2.66±0.13	17.7±1.21	6.65
17u	-		8.15±2.86	19.8±0.63	2.43
17v	-		3.31±0.29	7.26±0.28	2.19
17w	-		28.9±3.63	>50	/
17x	-		13.6±2.92	>50	/
17y	-		12.1±1.29	10.8±0.50	0.89
18a	-		1.12±0.17	9.96±1.33	8.89
18b	-		6.68±2.32	21.3±0.42	3.19
18c	-		10.3±2.79	23.2±0.43	2.25
18d	-		7.58±0.45	24.7±2.69	3.26
18e	-		8.40±1.87	26.7±0.57	3.18
22a	$-(CH_2)_2-$	H	27.6±4.07	20.8±1.6	0.75
22b	$-(CH_2)_2-$	-OH	6.15±0.12	7.97±2.21	1.30
22c	$-CH_2-$		5.14±1.76	11.5±1.61	2.24
22d	$-(CH_2)_3-$		7.65±0.31	2.91±0.28	0.38
22e	-		9.37±1.07	18.0±0.65	1.92
22f	-		7.72±2.75	6.39±0.37	0.83

[a]Incubation time for cytotoxic assay is 72 hours; [b] Resistance factor was calculated as a ratio of the IC_{50} value of KB/VCR cells to that of KB cells.

almost equivalent cytotoxic activities to compounds with ethyl-linker (e.g.**17s** and **17t**) and compounds with acetyl-linker (*e.g.* **17v**).

Although etoposide is widely used as therapy for cancer patients, the fact remains that tumors often acquire resistance to etoposide. Thus, with aim to determine whether there are drug resistance of compounds **17a-y**, **18a-e,** and **22a-f** as well as etoposide in multi-drug resistant cell lines (KB/VCR), resistance factor (RF), a ratio of cytotoxic activity against KB and KB/VCR of tested compounds were calculated as an evaluation criterion for their anti-resistance profiles. To our delight, most of the synthesized compounds, except for pyridine derivatives **17a,b** and glycosyl series **18a-e**, displayed superior cytotoxic activities against KB/VCR to that of etoposide, demonstrating that replacement of 4β-D-glucopyranose by a variety of disulfide/trisulfide-linked alkyl/heteroalkyl were beneficial for overcoming drug-resistance. In particular, RF values of several disulfide-containing derivatives **17j**, **17k**, **17l**, **17p** and **17q** (RF value: 1.73, 0.71, 0.33, 1.20 and 0.72 respectively) were significantly improved when comparing with etoposide (RF value: 7.40), while the cytotoxic activities of these compounds are more potent than that of etoposide. It should be noted that compound **17l** with piperidine moiety exhibited comparable cytotoxic activities against KB and significantly more potent cytotoxic activities against KB/VCR, with IC_{50} values of 2.90 μM (KB) and 0.97μM (KB/VCR), respectively. In addition, **22a-f** bearing trisulfide showed significantly more potent cytotoxic activities against KB/VCR with RF value ranging from 0.38 to 2.24, although they possessed comparable or slightly weaker cytotoxic activities against KB cells in comparison with etoposide. For example, compound **22d** exhibited remarkably sensitive cytotoxic activity against KB/VCR, which is almost 5.8-folds more potent than etoposide. It is revealed that tri-sulfide bond would be an excellent linker for chemical modification on 4β-podophyllotoxin to overcome the multi-drug resistant limitation of etoposide.

2.3 Metabolic stability study

To investigate the metabolic stability 4β-disulfide-containing podophyllotoxin derivatives, compound **17l** (the most promising compound) was chosen and undergone a preliminarily evaluation *in vitro* for the stability in human plasma. As shown in Fig.3, the concentration of compound **17l** was significantly decreased (c.a. 60%) in the first two hours, then reaching a stable state up to 8 hours. It is indicated that the disulfide would be stable in further *in-vivo* development, revealing a good prospect of these compounds for in-vivo anti-cancer activities.

Journal Name

ARTICLE

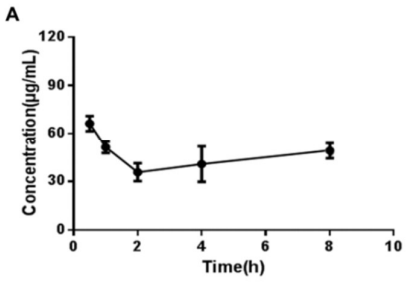


Fig.3. Metabolic stability in in vitro human plasma of Compound **17l**

3 Conclusion

Owing to some problems that still exists for practical use of etoposide in clinical settings, the development of a series of novel 4β-disulfide/trisulfide-4'-O-demethyl-4-desoxypodophyllotoxin derivatives was disclosed in present study. To be of interest, some of the compounds exhibited better cytotoxic activities than etoposide, not only for sensitive cancer cell lines (KB cells), but also for MDR cancer cell lines (KB/VCR cells). To our knowledge, this is the first time that biologically compatible disulfide/trisulfide bonds were introduced to the podophyllotoxin backbone. In addition to the excellent cytotoxic activities against KB and KB/VCR cells, **17l** has further shown remarkable results in the metabolic stability evaluation. Further *in-vitro* evaluation of drug likeness properties and *in-vivo* anticancer activity of **17l** are currently in progress in our laboratory.

4 Experimental section

4.1 Chemistry

Commercially available starting materials, reagents, and dry solvents were used as supplied. Melting points were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 500 MHz or 400 MHz, ^{13}C NMR were recorded on a 125 MHz and 100 MHz spectromter, respectively (chemical shifts are given in ppm (d) relative to TMS as internal standard, coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet, etc.). Mass spectral data were obtained on an Esquire-LC-00075 spectrometer. High resolution mass spectra were measured on an Agilent 1290 HPLC-6224 Time of Fight Mass Spectrometer. The synthesis of side chain intermediates **2-5**, **7**, **9**, **10**, **13**, **20**, and **21** were presented in Electronic Supplementary Material (ESI).

4.1.8General procedure for the synthesis of 17

Method A for compounds 17a-c, 17d', 17e-f and 17g': the commercial thiols (0.4 mmol) dissolved in CH_2Cl_2(2 mL) was added dropwise to a stirred solution of 1-chlorobenzotriazole(92 mg, 0.6 mmol) and benzotriazole (48 mg, 0.4 mmol) in CH_2Cl_2 (10 mL) under N_2 at -78 °C. The solution was allowed to warm to -20 °C and stirred for 2 hours. Compound 16 [17](250 mg, 0.6 mmol) was added slowly at -20 °C, and then allowed to warm to 0 °C for 3 hours. The reaction was quenched at 0 °C with a solution of $Na_2S_2O_3$ (0.05 g in 2 mL H_2O) and aq. $NaHCO_3$ (2 mL) with rapid stirring over 20 min, and then extracted with CH_2Cl_2 (3×50 mL).The combined organic layer was dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica column chromatography using DCM-EtOAc mixtures as eluent to give the desired product **17a-g**.

Method B for compounds 17h', 17i-m, 17n', 17o', 17p, 17q, 17r', 17s', 17t-u, 17v', 17w, 17x', 17y': Compound **3** or **5** (0.4 mmol) dissolved in CH_2Cl_2 (2mL) was added dropwise to a stirred solution of 1-chlorobenzotriazole (123 mg, 0.8 mmol) and benzotriazole (48 mg, 0.4 mmol) in CH_2Cl_2 (20 mL) under N_2 at -78 °C. The solution was stirred for 30 minutes and then added thiourea (91 mg, 1.2 mmol) with stirring for another 30 minutes. Compound **16**[17] (250 mg, 0.6 mmol) dissolved in CH_2Cl_2 (2mL) was added at -78 °C and the mixture was allowed to slowly warm to room temperature and stirred for 18 hours. The reaction was added water (100ml) and then extracted with CH_2Cl_2 (3×50 mL). The organic layer was washed with statured NaCl and dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using EtOAc–DCM mixtures as eluent to give the desired product **17h-y**.

Deprotection method for 17d', 17g'-h', 17s' and 17v': the obtained corresponding compound was immediately dissolved in DCM (5 mL), and HCl saturated EtOAc (1 mL) was added at 0 °C. The resulting mixture was allowed to warm to room temperature, and stirred until the disappearance of starting material. The mixture was evaporated, and the residue was added saturated $NaHCO_3$ aqueous solution, and extracted with DCM. The organic layer was washed with brine, and condensed *in vacuo* to give desired product.

Deprotection method for 17n', 17o', 17r' 17x'and 17y':the obtained corresponding compound was dissolved in CH_3CN (5 mL) and then was added $BF_3{\cdot}Et_2O$ (5 drops) in ice bath. After the disappearance of starting material, the mixture was evaporated *in vacuo.* The residue was added water and

extracted with DCM. After washed with brine, dried over anhydrous Na_2SO_4, the organic layer was evaporated to give desired product.

4.1.8.1 4β-(Pyridin-2-yldisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17a

Reagent: pyridine-2-thiol (44.4 mg, 0.4 mmol). The product was obtained as a white solid, yield:43%, mp: 214.8-216.2°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (d, *J* = 5.0 Hz, 1H, 6''-H), 7.81 (t, *J* = 6.0 Hz, 1H, 4''-H), 7.67 (d, *J* = 8.0 Hz, 1H, 3''-H), 7.30 (t, *J* = 5.0 Hz, 1H, 5''-H), 7.22 (s, 1H,5-H), 6.47 (s, 1H,8-H), 6.26 (s, 2H, 2', 6'-H), 5.96 (dd, *J* = 18.5, 1.5 Hz, 2H, -OCH_2O-), 4.78 (d, *J* = 4.0 Hz, 1H, 1-H), 4.59 (d, *J* = 5.0 Hz, 1H, 4-H), 4.55-4.48 (m, 2H, 11-H), 3.76 (s, 6H, 3',5'-OCH_3), 3.36 (dd, *J* =13.5, 5.0Hz, 1H, 2-H), 3.23-3.14 (m, 1H, 3-H); ESI-MS: m/z $[M+H]^+$ 526.

4.1.8.2 4β-(Pyridin-4-yldisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17b

Reagent: pyridine-4-thiol (44.4 mg, 0.4 mmol). The product was obtained as a white solid, yield: 55%, mp: 173.1-175.8°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, *J* = 4.0 Hz, 2H, pyridine), 7.50 (d, *J* = 5.5 Hz, 2H, pyridine), 6.81 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.24 (s, 2H, 2', 6'-H), 5.96 (d, *J* = 20.5 Hz, 2H, -OCH_2O-), 4.59 (d, *J* = 5.0 Hz, 1H, 1-H), 4.47 (d, *J* = 9.0 Hz, 2H, 11-H), 4.43 (d, *J* = 4.0 Hz, 1H, 4-H), 3.74 (s, 6H, 3',5'-OCH_3), 3.41 (dd, *J* = 14.0, 5.5 Hz, 1H, 2-H), 3.22-3.14 (m, 1H, 3-H);^{13}C NMR (125 MHz, $CDCl_3$) δ 174.14, 149.86, 148.72, 148.06, 147.38, 146.58, 134.33, 133.17, 130.75, 126.45, 120.89, 110.39, 110.10, 107.98, 101.85, 69.66, 56.56, 54.78,43.72, 41.86, 37.48; ESI-MS: m/z $[M+H]^+$ 526.

4.1.8.3 4β-(Thiophen-2-yldisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17c

Reagent: thiophene-2-thiol (46.4 mg, 0.4 mmol). The product was obtained as a white solid, yield:64%, mp: 223.2-225.1°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 5.5, 1.0 Hz, 1H,thiophene), 7.28 (dd, *J* = 3.5, 1.0 Hz, 1H, thiophene), 7.07 (dd, *J* = 5.5, 3.5 Hz, 1H, thiophene), 6.64 (s, 1H, 5-H), 6.43 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.94 (dd, 2H, *J* =14.0, 1.5 Hz, -OCH_2O-), 5.41 (s, 1H, -OH), 4.56 (d, *J* = 3.5 Hz, 1H, 1-H), 4.53 (d, *J* = 4.0 Hz, 1H, 4-H), 4.47-4.44 (m, 1H, 11-H), 4.36-4.33 (m, 1H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.23-3.18 (m, 2H, 2-H, 3-H) ;^{13}C NMR (125 MHz, $CDCl_3$) δ 174.52, 148.27, 147.27, 146.53, 134.23, 132.86, 131.01, 127.75, 110.31, 110.25, 108.06, 101.70, 70.08, 66.94, 57.82 , 56.57, 54.27, 53.63, 43.67, 41.82, 37.53, 36.67;ESI-MS: m/z $[M+H]^+$ 531.

4.1.8.4 4β-(L-AlanineN-[(1,1-Dimethylethoxy)carbonyl]methylester) -4'-O-demethyl-4-desoxy-podophyllotoxin 17d

Reagent: BocCysOMe (94 mg, 0.4 mmol).The product was obtained as a white solid in two steps, total yield: 44%,mp: 195.4-197.2 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1H, 5-H), 6.46 (d, *J* = 4.5 Hz, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (dd, *J* = 10.5 Hz,1.5Hz, 2H, -OCH_2O-), 4.55 (d, *J* = 5.0 Hz, 1H, 1-H), 4.51 (d, *J* = 4.5Hz, 1H, 4-H), 4.45-4.42 (m, 1H, 11-H), 4.40-4.36 (m, 1H, 11-H), 4.14 (s, 1H, 2''-H), 3.77 (s, 3H, 4''-H), 3.76 (s, 6H, 3',5'-OCH_3), 3.34 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), , 3.20-3.15 (m, 1H, 3-H), 3.13 (t, *J* = 4.5 Hz, 2H,1''-H); ESI-MS: m/z $[M+H]^+$ 550.

4.1.8.5 4β-Ethyldisulfanyl-4'-O-demethyl-4-desoxypodophyllotoxin 17e

Reagent: ethanethiol (25 mg, 0.4 mmol). The product was obtained as a white solid, yield: 61%, mp: 237.5-239.8°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.97 (dd, *J* = 12.0, 1.0 Hz, 2H, -OCH_2O-), 4.55 (d, *J* = 5.0 Hz, 1H, 1-H), 4.45-4.43 (m, 2H, 11-H), 4.39 (d, *J* = 4.5 Hz, 1H, 4-H), 3.78 (s, 6H,3',5'-OCH_3), 3.36-3.32 (m,1H, 2-H), 3.19-3.14 (m, 1H, 3-H), 2.82 (q, *J* = 7.5 Hz, 2H, 1''-H), 1.42 (t, *J* = 7.5 Hz, 3H, 2''-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.63, 148.25, 147.28, 146.50, 134.20, 132.75, 131.13, 127.94, 110.40, 110.23, 108.09, 101.69, 70.17, 56.60, 54.71, 43.70, 41.82, 37.58, 33.41, 14.54; ESI-MS: m/z $[M+H]^+$477.

4.1.8.6 4β-(2-Hydroxyethyl)disulfanyl)-4'-O-demethyl-4-desoxy podophyllotoxin 17f

Reagent: 2-mercaptoethanol (31.2 mg, 0.4 mmol). The product was obtained as a white solid, yield: 40%, mp: 221.9-223.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.99 (dd, *J* = 11.5, 1.5 Hz, 2H, -OCH_2O-), 4.56 (d, *J* = 5.0 Hz, 1H, 1-H), 4.48 (d, *J* = 4.5 Hz 1H, 4-H), 4.45-4.40 (m, 2H, 11-H), 4.02-3.96 (m, 2H, 2''-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.34 (dd, *J* = 14.0, 5.5 Hz, 1H, 2-H), 3.18-3.16 (m, 1H, 3-H), 2.98 (t, *J* = 5.0, 2H, 1''-H);^{13}C NMR (125 MHz, $CDCl_3$) δ 174.55, 148.36, 147.34, 146.53, 134.25, 132.87, 131.04, 127.59, 110.36, 110.28, 108.10, 101.74, 70.07, 60.51, 56.62, 54.66, 43.69, 42.16, 41.85, 37.56; ESI-MS: m/z $[M+H]^+$ 493.

4.1.8.7 4β-((2-Aminoethyl)disulfanyl)-4'-O-demethyl-4-desoxy podophyllotoxin 17g

Reagent:tert-butyl (2-mercaptoethyl)carbamate (70.8 mg, 0.4 mmol). The product was obtained as a white solid in two steps, total yield: 36%, mp: 193.3-194.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1H,5-H), 6.46 (s, 1H,8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (d, *J* = 4.5 Hz, 2H, -OCH_2O-), 4.55 (d, *J* = 5.0 Hz, 1H,1-H), 4.46-4.39 (m, 3H, 3-H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.34 (dd, *J* = 14.0, 5.5 Hz, 1H,2-H), 3.18-3.16 (m, 3H,3-H, 2''-H), 2.98-2.91 (m, 2H, 1''-H); ESI-MS: m/z $[M+H]^+$492.

4.1.8.8 4β-((3-(Piperazin-1-yl)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17h

Reagent:**3a** (104.8 mg, 0.4 mmol). The product was obtained as a white solid in two steps, total yield: 27%, mp: 179.8-181.6 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.12 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.19 (s, 2H, 2', 6'-H), 6.04 (d, *J* = 11.0 Hz, 2H, -OCH_2O-), 5.76 (s, 1H, -OH), 4.76 (d, *J* = 3.5 Hz, 1H, 1-H), 4.54 (t, *J* = 7.5 Hz, 1H, 11-H), 4.49 (d, *J* = 5.0 Hz, 1H, 4-H), 4.27 (t, *J* = 9.0 Hz, 1H, 11-H), 3.77-3.72 (m, 4H,piperazine), 3.63 (s, 6H, 3',5'-OCH_3), 3.30-3.24 (m, 7H, 2-H, 3''-H, piperazine), 3.10-3.02 (m, 2H, 1''-H), 2.98-2.90 (m, 1H, 3-H), 2.23-2.21 (m, 2H, 2''-H); ESI-MS: m/z $[M+H]^+$575.

4.1.8.9 4β-((3-Morpholinopropyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17i

Reagent:**3b** (64.4 mg, 0.4 mmol). The product was obtained as a white solid, yield: 34%, mp: 169.8-171.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.99 (d, *J* = 11.0 Hz, 2H,-OCH_2O-), 4.55 (d, *J* = 5.0 Hz, 1H, 1-H), 4.44-4.40 (m, 3H, 4, 11-H), 3.77 (s, 6H, 3',5'-OCH_3), 3.76-3.75 (m,4H,morpholine), 3.36 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H),

3.18-3.14 (m, 1H, 3-H), 2.86-2.79 (m, 2H, 3''-H), 2.59-2.48 (m, 6H, 1''-H, morpholine), 2.01-1.94 (m, 2H, 2''-H); ESI-MS: m/z $[M+H]^+$ 576.

4.1.8.10 4β-((N-Methylpiperazine-1-propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17j

Reagent: **3c** (69.6 mg, 0.4 mmol). The product was obtained as a yellow-white solid, yield: 33%, mp: 149.5-150.6°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (d, *J* = 11.0 Hz, 2H, -OCH_2O-), 5.30 (s, 1H, -OH), 4.55 (d, *J* = 5.0 Hz, 1H, 1-H), 4.44 (d, *J* = 9.0 Hz, 2H, 11-H), 4.41 (d, *J* = 4.0 Hz, 1H, 4-H), 3.77 (s, 6H，3',5'-OCH_3), 3.33 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.19-3.12 (m, 1H, 3-H), 2.86-2.80 (m, 2H, 3''-H), 2.64-2.47 (m, 10H, 1''-H,piperazine), 2.33 (s, 3H, -CH_3), 1.96-1.92 (m, 2H, 2''-H) ;^{13}C NMR (125 MHz, $CDCl_3$) δ174.57, 148.23, 147.24, 146.58, 134.27, 132.78, 131.02, 127.83, 110.42, 110.21, 108.09, 101.67, 70.10, 56.75, 56.57, 55.08, 54.43, 53.05, 45.98, 43.67, 41.81, 37.55, 26.38; ESI-MS: m/z $[M+H]^+$ 589.

4.1.8.11 4β-((3-(Pyrrolidin-1-yl)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17k

Reagent: **3d** (58.1 mg, 0.4 mmol). The product was obtained as a yellow-white solid, yield: 41%, mp: 159.4-160.7 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (s, 2H, -OCH_2O-), 4.55-4.53 (m, 2H, 1-H, 4-H), 4.47-4.38 (m, 2H, 11-H), 3.78 (s, 6H,3',5'-OCH_3), 3.35 (dd, *J* = 14.0, 5.5Hz, 1H, 2-H), 3.22-3.08 (m, 5H, 3-H, pyrrolidine), 3.03-2.98 (m, 2H, 3''-H), 2.93-2.86 (m, 2H, 1''-H), 2.39-2.22 (m, 2H, 2''-H), 2.14-2.08 (m, 4H, pyrrolidine); HR-MS (ESI+): calculated for $C_{28}H_{33}N_1O_7S_2$ $[M+H]^+$: 560.1771, found: m/z =560.1770.

4.1.8.12. 4β-((3-(Piperidin-1-yl)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17l

Reagent: **3e** (63.6 mg, 0.4 mmol). The product was obtained as a white solid, yield: 38%, mp: 153.2-155.5°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (dd, *J* = 10.0 Hz, 1.0Hz, 2H, -OCH_2O-), 5.30 (s, 1H, -OH), 4.55 (d, *J* = 5.5 Hz, 1H, 1-H), 4.46-4.42 (m, 3H, 4, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.36 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.20-3.12 (m, 1H, 3-H), 2.89-2.80 (m, 2H, 3''-H), 2.64-2.40 (m, 4H, piperidine), 1.76-1.44 (m, 10H, 1''-H, 2'', piperidine);^{13}C NMR (125 MHz, $CDCl_3$) δ 174.64, 148.25, 147.28, 146.53, 134.21, 132.77, 131.11, 127.88, 110.49, 110.21, 108.06, 101.69, 70.16, 57.61, 56.59, 54.71, 54.41, 43.71, 41.84, 37.80, 37.58, 29.83, 26.33, 25.89, 24.41;HR-MS (ESI+): calculated for $C_{29}H_{35}N_1O_7S_2$ $[M+H]^+$: 574.1928, found: m/z 574.1923.

4.1.8.13 4β-((3-(4-Acetylpiperazin-1-yl)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17m

Reagent: 3**f** (81.6 mg, 0.4 mmol). The product was obtained as a yellow-white solid, yield: 21%, mp: 159.8-161.5°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (dd, *J* = 9.0, 1.0 Hz, 2H,-OCH_2O-), 4.55 (d, *J* = 5.5 Hz, 1H, 1-H), 4.44 (br s, 1H, 4-H), 4.43 (dd, *J* = 10.0, 3.5 Hz, 2H, 11-H), 3.78 (s, 6H,3',5'-OCH_3), 3.72-3.59 (m, 2H,piperazine), 3.58-3.46 (m, 2H，piperazine), 3.36 (dd, *J* = 14.0, 5.5 Hz, 1H, 2-H), 3.20-3.12 (m, 1H, 3-H), 2.91-2.79 (m, 2H, 3''-H), 2.58-2.38 (m, 6H,piperazine, 1''-H), 2.10 (s, 3H, -CH_3), 2.02-1.91 (m, 2H, 2''-H);HR-MS (ESI+): calculated for $C_{30}H_{36}N_2O_8S_2$ $[M+H]^+$: 617.1986, found: m/z = 617.1987 $[M+H]^+$.

4.1.8.14 4β-((4-Piperidinolpropyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17n

Reagent: **3g** (44.4 mg, 0.4 mmol). The product was obtained as a red-white solid in two steps, total yield: 10%, mp: 235.5-236.3°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (dd, *J* = 9.5 Hz, 1.0Hz, 2H, -OCH_2O-), 4.55 (d, *J* = 5.0 Hz, 1H, 1-H), 4.44-4.42 (m, 3H, 4-H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.36 (dd, *J* = 14.0, 5.5 Hz, 1H, 2-H), 3.20-3.13 (m, 1H, 3-H), 3.12-3.06 (m, 2H,piperidine), 2.92-2.81 (m, 2H, 3''-H), 2.75-2.56 (m, 5H,piperidine), 2.32-2.20 (m, 2H, 2''-H), 2.10-2.00 (m, 2H, piperidine), 1.28-1.23 (m, 2H, 1''-H); HR-MS (ESI+): calculated for $C_{25}H_{39}N_1O_8S_2$ $[M+H]^+$: 590.1877, found: m/z = 590.1625$[M+H]^+$.

4.1.8.15 4β-((3-(2-(Hydroxymethyl)piperidin-1-yl)propyl)disulfanyl) -4'-O-demethyl-4-desoxypodophyllotoxin 17o

Reagent: **3h** (121.3 mg, 0.4 mmol). The product was obtained as a yellow-white solid in two steps, total yield: 12%, mp: 211.9-213.6°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.97 (d, *J* = 13.0 Hz, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.97 (s, 2H, -OCH_2O-), 5.30(s, 1H, -OH), 4.54 (d, *J* = 5.0 Hz, 2H, 1-H), 4.51 (d, *J* = 4.0 Hz, 1H, 4-H), 4.48 (dt, *J* = 19.0, 9.0 Hz, 2H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.37-3.29 (m, 1H, 2-H, CH_2OH,), 3.24-3.12 (m, 2H,CH_2OH, 3-H), 2.96-2.82 (m, 4H, 1'', 3''-H), 2.28-2.08 (m, 2H, 2''-H, piperdine), 1.88-1.72 (m, 6H, piperdine); ESI-MS: m/z $[M+H]^+$604.

4.1.8.16 4β-((3-(Diethylamino)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17p

Reagent: **3i** (58.8 mg, 0.4 mmol). The product was obtained as a white solid, yield: 26%, mp: 159.1-161.2°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (dd, *J* = 4.5, 1.0 Hz, 2H, -OCH_2O-), 4.55 (d, *J* = 5.5 Hz, 1H, 1-H), 4.49 (d, *J* = 4.5 Hz, 1H, 4-H), 4.45 (dd, *J* = 19.0, 8.0 Hz, 2H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.36 (dd, *J* = 14.0, 5.5 Hz, 1H, 2-H), 3.22-3.13 (m, 1H, 3-H), 2.93-2.84 (m, 8H, 1'', 3''-H, 4''-H, 4'''-H), 2.23-2.08 (m, 2H, 2''-H), 1.27 (t, *J* = 7.0 Hz, 6H, 5'',5'''-H)；^{13}C NMR (125 MHz, $CDCl_3$) δ 174.56, 148.34, 147.33, 146.53, 134.19, 132.86, 131.03, 127.63, 110.47, 110.23, 108.01, 101.74, 70.06, 56.59, 54.30, 50.70, 46.84, 43.70, 41.81, 37.54, 36.82, 10.27; HR-MS (ESI+): calculated for $C_{28}H_{33}N_1O_8S_2$ $[M+H]^+$:562.1928, found: m/z = 562.1919.

4.1.8.17 4β-((3-(Diisopropylamino)propyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17q

Reagent: **3j** (70.1 mg, 0.4 mmol). The product was obtained as a yellow-white solid, yield: 6%, mp: 167.7-169.3°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.27 (s, 2H, 2', 6'-H), 5.98 (s, 2H, -OCH_2O-), 4.56 (d, *J* = 5.5 Hz, 1H, 1-H), 4.53 (d, *J* = 4.5 Hz, 1H, 4-H), 4.47 (dt, *J* = 19.0, 9.0 Hz, 2H, 11-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.70-3.61 (m, 2H, 4'', 5''-H), 3.36 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.24-3.15 (m, 1H, 3-H), 3.11-3.02 (m, 2H, 3''-H), 2.97-2.82 (m, 2H, 1''-H), 2.59-2.46 (m, 2H, 2''-H), 1.57-1.40 (m, 12H, 6'',7'',8'', 9''-H); HR-MS (ESI+): calculated for $C_{30}H_{39}N_1O_7S_2$ $[M+H]^+$: 590.2241, found: m/z = 590.0812.

4.1.8.18 4β-((2-(3-Hydroxypiperidin-1-yl)ethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17r

Reagent: **5k** (110.1 mg, 0.4 mmol). The product was obtained as a white solid, and the corresponding product was dissolved in CH_3CN (5 mL) and $BF_3{\cdot}Et_2O$ (5 drops) was added with ice bath. After the disappearance of starting material, the mixture was evaporated *in vacuo* and the residue was added with DCM and H_2O. The organic layer was washed with saturated NaCl solution, dried over anhyd Na_2SO_4, and evaporated to give **17r** as a white solid, total yield: 28%, mp: 244.8-246.3°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (d, *J* = 3.6 Hz, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2'-H, 6'-H), 5.98 (d, *J* = 6.0 Hz, 2H,-OCH_2O-), 4.55 (dd, *J* = 5.0, 2.5 Hz, 1H, 1-H), 4.46-4.40 (m, 3H, 4-H, 11-H), 3.90-3.84 (m,piperidine), 3.78 (s, 6H, 3′,5′-OCH_3), 3.38-3.34 (m, 1H, 2-H), 3.20-3.12 (m, 1H, 3-H), 2.96-2.88 (m, 2H, 1′′-H), 2.82-2.67 (m, 2H, 1′′-H), 2.63-2.51 (m, 3H, piperidine), 2.43-2.35 (m, 1H, piperidine), 1.90-1.81 (m, 1H, piperidine), 1.66-1.52 (m, 3H, piperidine); ESI-MS: m/z $[M+H]^+$576.

4.1.8.19 4β-((2-(Piperazin-1-yl)ethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17s

Reagent:5**a** (98.5 mg, 0.4 mmol). The product was obtained as a white solid, total yield: 33%, mp: 194.5-195.4 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.19 (s, 2H, 2′, 6′-H), 6.04 (d, *J* = 12.0, 2H, -OCH_2O-), 4.76 (d, J = 3.5Hz, 1H, 1-H), 4.54-4.49 (m, 2H, 11-H), 4.27 (t, J = 9.0Hz, 1H, 2-H), 4.05 (q, J = 7.0 Hz, 1H, 3-H), 3.65-3.58 (m, 7H,4-H, 3′,5′-OCH_3), 3.46-3.18 (m, 12H,piperazine, 1'', 2''-H), 2.78-2.62 (m, 2H, 2''-H), 2.52-2.42 (m, 4H,piperazine); ESI-MS: m/z $[M+H]^+$ 561.

4.1.8.20 4β-((2-Morpholinoethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17t

Reagent: **5b** (58.8 mg, 0.4 mmol). The product was obtained as a white solid, yield: 42%, mp: 194.8-196.2°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2′, 6′-H), 5.98 (d, *J* = 11.5 Hz, 2H, -OCH_2O-), 5.55 (s, 1H, -OH), 4.56 (d, *J* = 3.5 Hz, 1H, 1-H), 4.50-4.38 (m, 3H, 4,11-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.74 (brs, 4H,morpholine), 3.37 (dd, *J* = 13.5, 4.5 Hz, 1H, 2-H), 3.21-3.10 (m, 1H, 3-H), 3.00-2.88 (m, 2H, 2′′-H), 2.82-2.65 (m, 2H, 1′′-H), 2.61-2.41 (m, 4H,morpholine); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.43, 148.31, 147.32, 146.52, 136.20, 134.57, 134.25, 132.81, 131.67, 130.98, 128.08, 127.47, 110.47, 110.07, 108.10, 101.65, 70.01, 56.64, 55.42, 43.68, 42.28, 37.36; ESI-MS: m/z $[M+H]^+$ 562.

4.1.8.21 4β-((2-Morpholino-2-oxoethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17u

Reagent: **7a** (64.4 mg, 0.4 mmol). The product was obtained as a white solid, yield: 69%, mp: 141.2-142.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (s, 1H,5-H), 6.44 (s, 1H,8-H), 6.27 (s, 2H, 2′, 6′-H), 5.97 (dd, *J* = 7.0, 1.0 Hz, 2H, -OCH_2O-), 5.30 (s, 1H,-OH), 4.75 (d, *J* = 4.5 Hz, 1H,1-H), 4.54 (d, *J* = 5.0 Hz, 1H, 4-H), 4.47 (dt, *J* = 19.0,9.0 Hz, 2H,11-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.75-3.67 (m, 8H, morpholine), 3.56-3.50 (m, 2H, 1′′-H), 3.30 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.21-3.15 (m, 1H, 3-H); HR-MS (ESI+): calculated for $C_{27}H_{29}N_1O_9S_2$ $[M+H]^+$: 576.1356, found: m/z = 575.9867$[M+H]^+$.

4.1.8.22 4β-((2-Oxo-2-(piperazin-1-yl)ethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17v

Reagent: **7b** (104.0 mg, 0.4 mmol). The product was obtained as a white solid, total yield: 36%, mp: 149.9-151.2°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (s, 1H, 5-H), 6.43 (s, 1H, 8-H), 6.26 (s, 2H, 2′, 6′-H), 5.96 (dd, *J* = 7.5, 1.0 Hz, 2H, -OCH_2O-), 4.74 (d, *J* = 4.0 Hz, 1H, 1-H), 4.52 (d, *J* = 5.0 Hz, 1H, 4-H), 4.46-4.36 (m, 2H, 11-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.73-3.68 (m, 4H,piperazine), 3.52-3.49 (m, 2H, 1′′-H), 3.29 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.20-3.12 (m, 1H, 3-H), 2.96-2.88 (m, 4H, piperazine); HR-MS (ESI+): calculated for $C_{27}H_{30}N_2O_8S_2$ $[M+H]^+$: 575.1516, found: m/z =575.0119$[M+H]^+$.

4.1.8.23 4β-((2-Oxo-2-(4-piperidone-1-yl)ethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17w

Reagent: **7c** (69.2 mg, 0.4 mmol). The product was obtained as a white solid, yield: 24%, mp: 155.3-156.8°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (s, 1H, 5-H), 6.45 (s, 1H, 8-H), 6.26 (s, 2H, 2'-H, 6'-H), 5.97 (d, *J* = 6.5 Hz, 2H, -OCH_2O-), 5.47 (s, 1H, -OH), 4.76 (d, *J* = 3.5 Hz, 1H, 1-H), 4.55 (d, *J* = 5.0 Hz, 1H, 4-H), 4.50-4.35 (m, 2H, 11-H), 3.90-3.84 (m, 2H, 1′′-H), 3.82-3.78 (m, 4H,piperidine), 3.76 (s, 6H, 3′,5′-OCH_3), 3.30 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.24-3.12 (m, 1H, 3-H), 2.62-2.50 (m, 4H, piperidine); ESI-MS: m/z $[M+H]^+$601.

4.1.8.24 4β-((2-(3-Hydroxypiperidin-1-yl)-2-oxoethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17x

Reagent: **7d** (115.6 mg, 0.4 mmol). The product was obtained as a white solid, total yield: 61%,mp: 189.3-190.2°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (m, 1H, 5-H), 6.43 (s, 1H, 8-H), 6.26 (d, *J* = 2.8 Hz, 2H, 2'-H, 6'-H), 5.96 (d, *J* = 5.5 Hz, 2H, -OCH_2O-), 5.43 (s, 1H, -OH), 4.81-4.74 (m, 1H, 1-H), 4.56-4.51(m, 1H, 4-H), 4.50-4.36 (m, 2H, 11-H), 3.91-3.83 (m, 7H,piperidine , 1′′-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.29-2.23 (m, 1H, 2-H), 3.18-3.12 (m, 1H, 3-H), 1.97-1.88 (m, 3H, piperidine), 1.73-1.65 (m, 1H, piperidine), 1.56 (d, *J* = 4.0 Hz, 1H, piperidine); ESI-MS: m/z $[M+H]^+$590.

4.1.8.25 4β-((2-(4-Hydroxypiperidin-1-yl)-2-oxoethyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 17y

Reagent:**7e** (115.6 mg, 0.4 mmol). The product was obtained as a white solid, total yield: 21%, mp: 194.5-196.1°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, *J* = 5.9 Hz, 1H, 5-H), 6.44 (s, 1H, 8-H), 6.27 (s, 2H, 2′, 6′-H), 5.96 (d, *J* = 6.5 Hz, 2H, -OCH_2O-), 4.76 (d, *J* = 4.0 Hz, 1H,1-H), 4.54 (d, *J* = 5.0 Hz, 1H,4-H), 4.46-4.39 (m, 2H, 11-H), 4.14-3.97 (m,2H, 1′′-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.77-3.74 (m, 2H,piperidine), 3.30 (dd, *J* = 14.0, 5.0 Hz, 1H, 2-H), 3.21-3.13 (m, 1H, 3-H), 1.98-1.88 (m, 2H, piperidine), 1.86-1.68 (m, 2H, piperidine), 1.68-1.49 (m, 3H,piperidine); ESI-MS: m/z $[M+H]^+$590.

4.1.9 General procedure for synthesis of 18a-c

To a solution of thiol-containing glycosyl derivates **13** (0.1mmol) in DCM/CH_3OH, and compound **17a** (52.5 mg, 0.1 mmol) in chloroform (2 mL) was added. The resulting mixture was stirred at room temperature overnight. Then, the solvent was evaporated *in vacuo*, and the residue was purified bycolumn chromatography on silica gel using DCM-Methanol as eluent to give the corresponding product.

4.1.9.1 4β-((1-Galactosyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 18a

Reagent:**13a** (19.6 mg, 0.1 mmol). The product was obtained as a white solid, yield: 73%; mp: 171.3-172.8°C; ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.25 (s, 1H, 5-H), 6.41 (s, 1H, 8-H), 6.30 (s,

2H, 2'-H, 6'-H), 5.94 (d, *J* = 2.5 Hz, 2H, -OCH_2O-), 5.02 (d, *J* = 4.0 Hz, 1H, 1'-H), 4.67 (d, *J* = 10.0 Hz, 1H, 1-H), 4.52-4.43 (m, 3H, 4-H, 11-H), 3.94 (d, *J* = 3.0 Hz, 1H, Gla), 3.89-3.84 (m, 1H, Gla), 3.81-3.73 (m, 2H, Gla), 3.72 (s, 6H, 3',5'-OCH_3), 3.68 (d, *J* = 9.5 Hz, 1H, Gla), 3.55 (dd, *J* = 9.0, 3.5 Hz, 1H, 2-H) , 3.39-3.32 (m, 1H, Gla), 3.27-3.18 (m, 1H, 3-H)；^{13}C NMR (125 MHz, MeOD) δ 175.76, 148.15, 147.16, 147.05, 134.48, 132.44, 131.04, 128.55, 110.41, 109.27, 108.16, 101.43, 93.98, 79.93, 74.89, 70.31, 69.80, 69.16, 61.49, 55.43, 55.30, 43.41, 41.39, 37.74; ESI-MS: m/z $[M+H]^+$611.

4.1.9.2 4β-((1-Glucosyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 18b

Reagent: **13c** (19.6 mg, 0.4 mmol). The product was obtained as a white solid, yield: 59%; mp: 175.6-176.6℃; ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.30 (s, 1H, 5-H), 7.14 (s, 1H, -OH), 6.46 (s, 1H，8-H), 6.31 (s, 2H, 2'-H, 6'-H), 6.00 (s, 2H, -OCH_2O-), 5.11 (d, *J* = 3.0 Hz, 1H, 1'-H), 4.78 (d, *J* = 9.5 Hz, 1H, 1-H), 4.59 (d, *J* = 5.0 Hz, 1H，4-H), 4.56-4.54 (m, 2H, glu), 4.46-4.44 (m, 1H, 11-H), 4.36-4.31 (m, 2H, glu, 11-H), 3.97 (d, *J* = 2.5 Hz, 1H, glu), 3.88-3.83 (m, 1H, glu), 3.77-3.71 (m, 1H, glu), 3.68 (s, 6H, 3',5'-OCH_3), 3.60-3.56 (m, 1H, glu), 3.54-3.49 (m, 1H, glu), 3.48-3.40 (m, 2H, glu), 3.29-3.26 (m, 2H, 2-H, 3-H), 3.18-3.12 (m, 1H, glu) ; ^{13}C NMR (125 MHz, Acetone-d_6) δ 174.80, 148.91, 147.88, 147.83, 136.09, 133.64, 131.81, 129.64, 111.52, 110.36, 109.69, 102.39, 93.55, 82.26, 79.60, 73.78, 71.40, 70.46, 62.85, 56.66, 56.35, 44.33, 42.03, 38.37; ESI-MS: m/z $[M+NH_4]^+$628.

4.1.9.3 4β-((1-Acetyl galactosyl)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 18c

Reagent: **13b** (36.4 mg, 0.4 mmol). The product was obtained as a white solid, yield: 51%; mp: 149.1-150.6℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.46 (s, 1H), 6.27 (s, 2H), 5.97 (dd, *J* = 7.5, 1.0 Hz, 2H), 5.51 (d, *J* = 3.0 Hz, 1H), 5.29 (t, *J* = 10.0 Hz, 1H), 5.10 (dd, *J* = 10.0, 3.5 Hz, 1H), 4.81 (d, *J* = 10.0 Hz, 1H), 4.77 (d, *J* = 4.0 Hz, 1H), 4.53 (d, *J* = 5.0 Hz, 1H), 4.47 (dd, *J* = 8.5, 7.0 Hz, 1H), 4.37 (t, *J* = 9.5 Hz, 1H), 4.28 (dd, *J* = 11.5, 7.0 Hz, 1H), 4.23-4.18 (m, 1H), 4.16-4.08 (m, 2H), 3.77 (s, 6H), 3.24-3.12 (m, 2H), 2.20 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H); ESI-MS: m/z $[M+Na]^+$801.

4.1.10 4β-((2-(Thiophen-2-yl)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diol)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 18d

To a solution of compound **18b** (68 mg, 0.11 mmol) in 2-thenaldehyde (1 mL), dry $ZnCl_2$ (34 mg) was added under N_2 protection. After the reaction completed, the mixture was added water and extracted with DCM. The combined organic layer was washed with brine and condensed *in vacuo*. The residue was purified by column chromatography on silica gel using DCM-EtOAc as eluent to give **18d** as a white solid, yield: 39%; mp: 168.7-170.2^{o}C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.48 (dd, *J* = 5.0, 1.0 Hz, 1H,thiophene), 7.19 (d, *J* = 3.0 Hz, 1H, thiophene), 7.17 (s, 1H, 5-H), 7.14 (s, 1H, -OH), 7.03 (dd, *J* = 5.0, 3.5 Hz, 1H, thiophene), 6.49 (s, 1H, 8-H), 6.33 (s, 2H, 2'-H, 6'-H), 6.02 (dd, *J* = 3.0, 1.0 Hz, 2H, -OCH_2O-), 5.93 (s, 1H, 7'-H), 4.92-4.88 (m, 4H, Glu), 4.56 (d, *J* = 4.0 Hz, 1H, 1-H), 4.50-4.46 (m, 1H, 4-H), 4.39-4.31 (m, 2H, 11-H), 3.85 (t, *J* = 10.1 Hz, 1H, Glu), 3.81-3.72 (m, 2H,Glu), 3.69 (s, 6H, 3',5'-OCH_3), 3.66-3.62 (m, 1H,Glu), 3.59 (t, *J* = 9.5 Hz, 1H, Glu), 3.34-3.29 (m, 2H, 2-H, 3-H); ^{13}C NMR (125 MHz, Acetone) δ 174.67, 149.01, 147.92, 147.90, 141.50, 136.10, 133.78, 131.66, 129.65, 127.03, 126.76, 126.61, 110.77, 110.53, 109.65, 102.51, 99.08, 92.79, 81.60, 75.75, 74.28, 71.41, 70.35, 69.03, 56.72, 56.64, 44.33, 42.04, 38.21; ESI-MS: m/z $[M+H]^+$705.

4.1.11 4β-((2-Methylhexahydropyrano[3,2-d][1,3]dioxine-7,8-diol)disulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 18e

To a solution of compound **18b** (37 mg, 0.06 mmol) and TsOH (10 mg) in acetonitrile(10 mL), 1,1-Dimethoxyethane (0.15 mL,1.43 mmol) was added under N_2 protection. The mixture was allowed to stir at room temperature for 3 hours. After the reaction completed, the mixture was added water and extracted with DCM. The combined organic layer was washed with brine and condensed *in vacuo*. The residue was purified by column chromatography on on silica gel using DCM-EtOAc as eluent to give the corresponding product **18e** as a white solid, yield: 69%; mp: 163.2-164.7^{o}C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.14 (s, 1H, 5-H), 7.07 (s, 1H, -OH), 6.48 (s, 1H, 8-H), 6.33 (s, 2H, 2'-H, 6'-H), 6.01 (d, *J* = 1.5 Hz, 2H, -OCH_2O-), 4.87 (d, *J* = 2.0 Hz, 1H, Glu), 4.82 (d, *J* = 9.5 Hz, 1H, Glu), 4.79-4.76 (m, 2H, Glu), 4.70 (d, *J* = 4.5 Hz, 1H, 1-H), 4.54 (d, *J* = 4.0 Hz ,1H, 4-H), 4.48-4.44 (m, 1H, 11-H), 4.36-3.31 (m, 1H, 11-H), 4.22-4.18 (m, 1H, 7'-H), 3.69 (s, 7H, 3',5'-OCH_3, Glu), 3.60-3.53 (m, 3H, Glu), 3.35-3.28 (m, 3H, Glu, 2-H, 3-H), 1.27 (d, *J* = 5.0 Hz, 3H, -CH_3) ; ESI-MS: m/z $[M+H]^+$637.

4.1.15 General procedure for synthesis of 22a-f

Method for compound 22a-e and 22f': The mixture of compound **16**[17] (83mg, 0.2 mmol) and 21 (0.24 mmol) in DCM (5 mL) was stirred at room temperature for 3 hours under N_2 protection. Then, the solvent was evaporated in vacuo, the residue was purified by column chromatography on silica gel using PE-DCM-EtOAc as eluent to give the desired product.

Deprotection method for 22f: The obtained **22f'** was immediately dissolved in DCM (5 mL), and HCl saturated EtOAc (1 mL) was added at 0 °C. The mixture was allowed to stir at room temperature until the starting material disappeared. Then, the solvent was evaporated, added $NaHCO_3$ saturated aqueous solution, and extracted with DCM. The combined organic layer was washed with bine, dried over anhydrous Na_2SO_4, evaporated in vacuo to give compound **22f** without purification.

4.1.15.1 4β-Ethyltrisulfanyl-4'-O-demethyl-4-desoxypodophyllotoxin 22a

Reagent: **21a** (57.4 mg, 0.24 mmol). The product was obtained as a white solid, yield: 34%, mp: 243.5-244.3^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s, 1H, 5-H), 6.45 (s, 1H, 8-H), 6.28 (s, 2H, 2'-H, 6'-H), 5.60 (dd, *J* = 14.0, 1.5 Hz, 2H, -OCH_2O-), 5.42 (s, 1H, -OH), 4.64 (d, *J* = 3.5 Hz, 1H, 1-H), 4.55 (d, *J* = 4.0 Hz, 1H, 4-H), 4.50-4.40 (m, 2H, 11-H), 3.78 (s, 6H,3',5'-OCH_3), 3.24-3.15 (m, 2H, 2-H, 3-H), 3.05 -2.93 (m, 2H, 1''-H), 1.44 (t, *J* = 7.5 Hz, 3H, 2''-H); ESI-MS: m/z$[M+Na]^+$531.

4.1.15.2 4β-((2-Hydroxyethyl)trisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 22b

Reagent: **21b** (61.2 mg, 0.24 mmol). The product was obtained as a white solid, yield: 59%, mp: 229.3-231.1^{o}C; ^{1}H NMR (500

MHz, $CDCl_3$) δ 6.94 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.28 (s, 2H, 2'-H, 6'-H), 5.99 (dd, *J* = 14.0, 1.5 Hz, 2H, -OCH_2O-), 5.43 (s, 1H, -OH), 4.67 (d, *J* = 4.0 Hz, 1H, 1-H), 4.55 (d, *J* = 4.5 Hz, 1H, 4-H), 4.47 (t, *J* = 7.0 Hz, 1H, 11-H), 4.40 (t, *J* = 9.5 Hz, 1H, 11-H), 4.01 (t, *J* = 5.5 Hz, 2H, 1''-H), 3.78 (s, 6H,3',5'-OCH_3), 3.26-3.08 (m, 4H, 2-H, 3-H, 2''-H); ESI-MS: m/z $[M+H]^+$ 525.

4.1.15.3 4β-((2,3-Dihydroxypropyl)trisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 22c

Reagent: **21c** (68.4 mg, 0.24 mmol). The product was obtained as a white solid, yield: 36%, mp: 251.5-252.9°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (d, *J* = 23.0 Hz, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.28 (s, 2H, 2'-H, 6'-H), 5.99 (d, *J* = 10.5 Hz, 2H, -OCH_2O-), 5.43 (s, 1H, -OH), 4.67 (d, *J* = 3.5 Hz, 1H, 1-H), 4.56 (d, *J* = 3.5 Hz, 1H, 4-H), 4.47 (d, *J* = 6.5 Hz, 1H, 11-H), 4.40-4.36 (m, 2H, 11, 3''-H), 3.86-3.83 (m, 1H, 3''-H), 3.78 (s, 6H,3',5'-OCH_3), 3.69-3.63 (m, 1H, 2'-H), 3.25-3.00 (m, 4H, 2, 3, 1''-H); HR-MS (ESI^+): calculated for $C_{23}H_{24}O_7S_3$ $[M+NH_4]^+$: 572.1077, found: m/z = 572.1084.

4.1.15.4 4β-((3-Morpholinopropyl)trisulfanyl) -4'-O-demethyl-4-desoxypodophyllotoxin 22d

Reagent: **21d** (81.1 mg, 0.24 mmol). The product was obtained as a white solid, yield: 38%, mp: 180.3-181.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 1H, 5-H), 6.39 (s, 1H, 8-H), 6.20 (s, 2H, 2'-H, 6'-H), 5.92 (dd, *J* = 8.4, 1.2 Hz, 2H, -OCH_2O-), 4.48 (d, *J* = 5.2 Hz, 1H, 1-H), 4.38-4.33 (m, 3H, 4-H, 11-H), 3.71 (s, 6H,3',5'-OCH_3), 3.70-3.64 (m, 4H, morpholine), 3.29 (dd, *J* = 13.8, 5.2 Hz, 1H, 2-H), 3.13-3.03 (m, 1H, 3-H), 2.83-2.72 (m, 2H, 2''-H), 2.48-2.34 (m, 6H,morpholine, 3''-H), 1.94-1.84 (m, 2H, 1''-H);HR-MS (ESI^+) calculatedfor $C_{28}H_{33}N_1O_8S_3$ $[M+H]^+$: 608.1441, found: m/z =608.1445 $[M+H]^+$.

4.1.15.5 4β-(((2R,3S,4R,5R,6S)-3,4,5-Trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)trisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 22e

Reagent: **21e** (89.5 mg, 0.24 mmol). The product was obtained as a white solid, yield: 9 %, mp: 182.9-184.8°C; ^{1}H NMR (400 MHz, Acetone) δ 7.30 (s, 1H, 5-H), 7.09 (s, 1H, Phenol), 6.46 (s, 1H, 8-H), 6.32 (s, 2H, 2'-H, 6'-H), 5.99 (s, 2H, -OCH_2O-), 5.11 (d, *J* = 1.6 Hz, 1H, 1'-H), 4.77 (d, *J* = 9.6 Hz, 1H, 1-H), 4.61-4.51 (m, 3H, 4-H,glu), 4.50-4.43 (m, 1H, 11-H), 4.39-4.28 (m, 1H, 11-H), 4.32 (d, *J* = 4.5 Hz, 1H, glu), 4.01 (ddd, *J* = 11.5, 5.0, 2.5 Hz, 1H, glu), 3.85-3.80 (m, 1H, glu), 3.78-3.74 (m, 1H, glu), 3.68 (s, 6H, 3',5'-OCH_3), 3.60-3.39 (m, 5H, glu), 3.30-3.27 (m, 2H, 2, 3-H); HR-MS (ESI+) calculatedfor $C_{27}H_{30}O_{12}S_3$ $[M+H]^+$: 643.0972, found: m/z=643.0968$[M+H]^+$.

4.1.15.64β-((2-Oxo-2-(piperazin-1-yl)ethyl)trisulfanyl)-4'-O-demethyl-4-desoxypodophyllotoxin 22f

Reagent: **21f** (69.2 mg, 0.4 mmol). The product was obtained as a white solid, two steps yield: 41%, mp: 151.8-153.6°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H, 5-H), 6.45 (s, 1H, 8-H), 6.28 (s, 2H, 2'-H, 6'-H), 5.99 (dd, *J* = 5.0, 1.0 Hz, 2H, -OCH_2O-), 4.77 (d, *J* = 4.0 Hz, 1H, 1-H), 4.56 (d, *J* =5.0 Hz, 1H, 4-H), 4.49 (t, *J* = 9.5 Hz, 1H, 11-H), 4.38 (t, *J* = 9.5 Hz, 1H, 11-H), 3.90 (q, *J* =14.0 Hz, 2H, 1''-H), 3.78 (s, 6H, 3',5'-OCH_3), 3.75-3.69 (m, 2H, piperazidine), 3.61- 3.57 (m, 2H,piperazidine), 3.24-3.18 (m, 2H, 2, 3-H), 3.02-2.93 (m, 4H,piperazidine), 2.06 (s, 1H, *N*-H); ESI-MS: m/z $[M+H]^+$607.

4.2 Cytotoxic activities assay

The cytotoxic activities of the tested compounds in KB and KB/VCR cells were measured using the SRB (sulforhodamine B) method. Cells were seeded in 96-well microtiter plates (at a density of 4000 cells per well) for overnight attachment and exposed to each of the test compound (1.0 ~ 100.0 μM) for 72 h. The SRB solution (5.0 mg/mL in RPIM 1640 medium; Sigma-Aldrich) was added (20.0 μl/well), and plates were incubated for a further 4 h at 37°C. The purple formazan crystals were dissolved in 100.0 μL of DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 490 nm. Assays were performed in triplicate and independently for three times. The concentration of drug inhibiting 50% of cells (IC_{50}) was calculated using the software of dose-effect analysis.

4.3 Evaluation of metabolism stability in human plasma

Compound **17l** (2 mg) was dissolved in DMSO (20μL) and acetonitrile (180μL) respectively, and then they were diluted with phosphate buffer saline (PH = 7.4) to afford stock solution (2 mg/mL, 1 mL). Human plasma was incubated at 37 °C for 15min. An aliquot (100 μL) of the stock solution was added to the human plasma (100 μL), and the mixture was incubated at 37 °C. At different time intervals (maximum incubation = 8h), the reaction was stopped by addition of 200μL acetonitrile containing nitrobenzene (internal standard). After precipitation of proteins, the samples were centrifuged at 14000 rpm for 8 min to pellet the precipitated protein. Then, the supernatant (20 μL) of these aliquots was analysed *via* HPLC to determine the residual amount of **17l**. The absorbance detector was set at a wavelength of 254nM, and the mobile phase consisted of acetonitrile: water (containing 0.1% trifluoroacetic acid) = 40:60 (v/v %). The mobile phase flow rate was 1.0 mL/min, and the HPLC column (250 mm × 4.6 mm) was packed with 5 μm C18.

Acknowledgements

We thank Mrs. Jianyang Pan (Pharmaceutical Informatics Institute, Zhejiang University) for performing the NMR spectrometry. Zhejiang Provincial Natural Science Foundation of China (LY13H300002) and Program for Zhejiang Leading Team of S&T Innovation (2011R50014).

Notes and references

1 a) P.J. Hogg, *Trends Biochem. Sci.*, 2003, **28**, 210-214; b) N. Nagahara, *Amino Acids,* 2011, **41**, 59-72; M. A. c) Wouters, S. W. Fan, N. L. Haworth, *Antioxid. Redox Signal.* 2010, **12**, 53-91.

2 a) T. Chatterji, K. Keerthi, K.S. Gates, *Bioor.Med. Chem. Lett.*, 2005, **15**, 3921-3924; b) C. M. Cremers, U.Jakob, *J. Biol. Chem.*2013, **288**, 26489-26496; c) L.-J. Yan, *Oxid. Med. Cell.Longev.*2014, 1-12.

3 a) I. Ojima, *Acc. Chem. Res.*, 2008, **41**, 108-119; b) J. Wang, S. Li, T. Luo, C. Wang, J. Zhao, *Curr. Med. Chem.* 2012, **19**, 2976-2983; K. FitzGerald, P. Holliger, G. Winter,*Protein Eng.* 1997, **10**, 1211-1225.

4 a) S.H. Lee, *Arch. Pharm. Res.*, 2009, **32**, 299-315;b) C. S. Jiang, W. E. Müller, H. C. Schröder, Y. W. Guo, *Chem. Rev.*, 2012,**112**, 2179-2207.

5 A. Szilagyi, F. Fenyvesi, O. Majercsik, I. F. Pelyvas, I. Bacskay, P. Fehér, J. Varadi, M. Vecsernyés, P. Herczegh, *J. Med. Chem.*,2006, **49**, 5626-5630.

6 M.Tomasz,Topics in molecular and structural biology: molecular aspects of anticancer drug-DNA interactions.vol. 2, Neidle, S., and Waring, M. (Eds.), Macmillan, New York, 1994, 312-347.

7 B. S.Davidson, T. F. Molinski, L. R. Barrows, C. M.Ireland, *J. Am. Chem. Soc.*, 1991, **113**, 4709-4710.

8 J.Golik, J. Clardy, G.Dubay, G.Groenewold, H.Kawaguchi, M.Konishi,B. Krishnan, H.Ohkuma,K. Sithoh, T. W.Doyle, *J. Am. Chem. Soc.*, 1987, **109**, 3461-3462.

9 a) D. M. Vyas, Y. Chiang, D. Benigni, W. C. Rose, W. T. Brander, *Recent advances in chemotherapy. Anticancer section.* Tshigami, J. (Ed.), University of Tokyo Press, Tokyo, 1985, 485-486; b) M. Kono, Y. Saitoh, M. Kasai, A. Sato, K. Shirahata, M. Morimoto, T. Ashizawa, *Chem. Pharm. Bull.*, 1989, **37**, 1128-1130.

10 P. Meresse, E. Dechaux, C. Monneret, E. Bertounesque, *Curr.Med.Chem.*, 2004, **11**, 2443-2466.

11 K. Seiter, *Expert Opin. Drug Saf.*, 2005, **4**, 219-234.

12 R.M. Moreas, F.E. Dayan, C. Canel, *Stud. Nat. Prod. Chem.,*2002, **26**, 149-182.

13 T. Utsugi, J. Shibata, Y. Sugimoto, K. Aoyagi, K. Wierzba, T. Kobunai, T. Terada, T. Oh-hara, T. Tsuruo, Y. Yamada, *Cancer Res.*, 1996, **56**, 2809-2814.

14 T.S. Huang, C.H. Shu, W.K. Yang, J. Whang-Peng,*Cancer Res.*, 1997, **57**, 2974-2978.

15 I. Rassmann, R. Thodtmann, M. Mross, A. Huttmann, W.E. Berdel, C. Manegold, H.H. Fiebig, A. Kaeser-Frohlich, K. Burk, A.R. Hanauske, *Invest. new drug*, 1998, **16**, 319-324.

16 L. Wang, F. Yang, X. Yang, X. Guan, C. Hu, T. Liu, Q. He, B. Yang, Y. Hu, *Eur.J.Med.Chem.*, 2001,**46**, 285-296.

17 Z. Wang, W. Ma, C.Zhang, *Huaxue Xuebao*,1992, **50**, 698-701.

18 N. Stellenboom, R. Hunter, M.R. Caira, *Tetrahedron*, 2010, **66**, 3228-3241.

19 M.V.Kalnins, *Can. J. Chem.*, 1966, **44**, 2111-2113.

20 A.L. Smith, C.K. Hwang, E. Pitsinos, G.R. Scarlato, K.C. Nicolaou, *J. Am. Chem.Soc.*,1992, **114**, 3134-3136.

Graphic abstract:

A novel series of podophyllotoxin derivatives bearing 4β-disulfide/trisulfide were designed, synthesized and biologically evaluated for their cytotoxic activities against KB cells and KB/VCR cells.